

ORIGINAL ARTICLE

Contribution of 1555 A-G mutation testing in patients with hereditary hearing loss

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ABSTRACT

Hypothesis: Sensorineural hearing impairment with mitochondrial inheritance is a major cause of hereditary hearing loss and the most important clinical cause, as the 1555 A-to-G mutation in mitochondrial 12SrRNA causes aminoglycoside-induced ototoxicity, as well as major phenotype abnormalities.

Material and methods: We retrospectively reviewed the records of 15 patients with bilateral, postlingual, sensorineural hearing impairment and a pattern of familial involvement consistent with mitochondrial inheritance. The patients were screened for the A1555G mutation in mitochondrial DNA.

Results: Peripheral blood screening tests identified the A1555G mutation in 8 patients, including 4 with a history of aminoglycoside therapy. High-frequency hearing loss was the most common audiometric pattern.

Conclusion: The A1555G mutation may be a common cause of hearing impairment. In patients with familial sensorineural hearing impairment, a high level of suspicion for mitochondrial inheritance should be maintained to ensure that appropriate genetic tests are done and that aminoglycosides are not given to carriers of the mutation whose hearing is still normal.

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Keywords: Sensorineural hearing impairment, Mitochondrial DNA, Aminoglycosides, Mutation.

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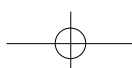
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INTRODUCTION

Sensorineural hearing impairment (SNHI) is common, and about two thirds of cases of hearing loss in childhood are due to genetic factors. The causes of inherited hearing impairment are extremely diverse [1-2].

Mutations in mitochondrial DNA (mtDNA) can cause both syndromic SNHI, for instance in MELAS syndrome (mitochondrial encephalopathy with lactic acidosis and stroke-like episodes) or Kearns-Sayre syndrome, and nonsyndromic SNHI [3-4]. Mutations in mtDNA responsible for nonsyndromic SNHI have been identified in the tRNA Ser (UCN) gene (T7510C [5] and T7511C [6]) and in the 12S rRNA gene (T1095C [7] and A1555G [8]). Additional mutations in mtDNA can cause either syndromic or nonsyndromic SNHI; they include the A7445G mutation in the nucleotide adjacent to the tRNA Ser (UCN) gene [9-10]. Another example is 7472insC (insertion of a cytosine at position 7472) in the tRNA Ser (UCN) gene, which causes either SNHI with ataxia, myoclonus, and arthropathy [11] or isolated SNHI [12].

The A1555G mutation may be the most common mitochondrial mutation [13]. It results in substitution of a guanine for an adenine in the mtDNA gene coding for 12S ribosomal RNA. In most cases, the A1555G mutation is associated with nonsyndromic SNHI, although one family had SNHI with SNHI with myocardiopathy [14].

The prevalence of the A1555G mutation in Europeans with SNHI varies across studies from 0.5% to 2.4% [15-18]. In Spanish families with nonsyndromic SNHI, however, prevalences of 15% [19], 27.1% [20], and 28.5% [21] have been reported. On the other hand, no cases of A1555G were found in 106 patients with SNHI in Greece [22] or 202 patients with early-onset nonsyndromic SNHI in the UK [23].

Thus, the true prevalence of the A1555G mutation remains unclear. Abundant evidence demonstrates that the mtDNA A1555G mutation induces susceptibility to hearing loss caused by exposure to aminoglycosides [8, 20, 24-28] in dosages that are usually safe for the cochlea. However, SNHI in patients with the mutation can develop in the absence of aminoglycoside exposure [5, 20, 28]. The auditory phenotype associated with the mutation varies widely, from normal audition to early-onset profound hearing loss.

The objective of this study was to determine the prevalence of the A1555G mutation in the 12S rRNA gene of mtDNA in patients with bilateral postlingual SNHI having a pattern of matrilinear transmission consistent with mitochondrial inheritance.

PATIENTS AND METHODS

Patients and families

We retrospectively reviewed the medical records of patients managed at the Vall d'Hebron General Hospital in Barcelona for familial SNHI. We looked for patients with nonsyndromic, postlingual, bilateral SNHI affecting at least two members of the family and having a pattern of transmission consistent with mitochondrial inheritance. Patients with SNHI due to a nongenetic cause other than aminoglycoside exposure were not considered for the study. We identified 15 patients meeting our inclusion criteria. The patients were Caucasians from 15 separate families whose ancestors came from various geographic regions, although all 15 patients resided in the same region. There were 12 (80%) women and 3 (20%) men, and mean age was 46 years (range, 19-78 years).

Methods

We established the pedigree of each patient. A history of aminoglycoside exposure or of tinnitus or vertigo was sought and recorded. Each patient underwent an otoscopic examination and liminal tonal audiometry. Hearing impairment was defined as an inability to hear pure-tone sounds of less than 30 dB in any of the frequencies tested. Severity was determined according to American Medical Association criteria [29] as mild (21-39 dB), moderate (40-69 dB), severe (70-89 dB), or profound (>90 dB).

Mitochondrial DNA tests

Informed consent to mtDNA testing was obtained in writing from each study participant prior to collection of a peripheral blood sample. Total DNA was extracted from the sample using phenol/chloroform/isoamyl-alcohol. PCR amplification was performed using the forward primer nt 1450-1470 and the reverse primer nt 1721-1741. The PCR fragment containing the 12S rRNA gene was sequenced using the ABI Prism 410 sequencer (Applied Biosystems Inc, Foster City, CA). The DNA sequence was examined manually and automatically for the A1555G mutation by comparing nucleotide sequences to a reference human mtDNA (Figure 1).

RESULTS

Of the 15 study participants, 8 (53.33%) had the A1555G mutation in the 12S rRNA gene of mtDNA. Four of these

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patients had a history of aminoglycoside use. The number of individuals with hearing loss in each of the 15 families ranged from 2 to 19 (mean, 6.8) (Table I). The hearing loss was mild in 2 patients, moderate in 2 patients, severe in 4 patients, and profound in 7 patients (Table II). None of the 8 patients with the A1555G mutation had mild or moderate hearing loss: 1 had severe and 7 profound hearing loss. All 4 patients with the A1555G mutation and aminoglycoside exposure had profound hearing loss. In 2 of these 4 patients, the age at aminoglycoside was known (6 and 17 years, respectively), and hearing loss occurred with the first dose. In the other 2 patients, hearing loss occurred at 5 and 16 years of age, respectively, but the timing relative to aminoglycoside use was not known. Age at hearing loss onset was not known in the 4 patients with the mutation but no known history of aminoglycoside use. High frequencies were predominantly affected. Two of these patients reported continuous bilateral tinnitus, and both established a link between aminoglycoside exposure and the onset of tinnitus. Four patients reported a history of peripheral vertigo; among them, 2 had been exposed to aminoglycosides but established no link between this exposure and the vertigo. These data suggest that aminoglycoside ototoxicity may not cause acute vestibular damage in patients who have the A1555G mutation.

DISCUSSION

The transmission of mtDNA occurs only through the mother. Males receive mtDNA mutations present in the maternal mtDNA but do not transmit it to their offspring.

The prevalence of mitochondrial mutations varies considerably across countries, and no comparative studies are available. We found the mutation in 8 (53.3%) of 15

patients with nonsyndromic, bilateral, postlingual SNHI. High prevalences of 15% to 30% have been reported in patients with nonsyndromic familial SNHI in Spain [21-23]. The higher prevalence in our study is ascribable to the fact that we included only patients whose pedigrees suggested a matrilineal pattern of inheritance.

The aminoglycoside class of antibiotics was introduced on the market in the 1950s. Aminoglycosides are inexpensive and remain first-line agents for the treatment of common infections, including tuberculosis. They are widely used for postsurgical prophylaxis and for treating surgical wound infections. Many studies have established that aminoglycosides are ototoxic in patients who have the mtDNA A1555G mutation [8, 20, 24-28].

Table I: Results

Family	Age (years)	Sexe	Nb of relatives with SNHI	AG exposure	A1555G +
1	47	F	8	yes	yes
2	57	M	4	no	no
3	45	F	4	no	no
4	72	F	3	no	no
5	43	F	4	no	no
6	78	F	5	no	yes
7	75	F	3	no	no
8	47	F	16	no	yes
9	51	M	5	yes	yes
10	36	M	6	yes	yes
11	33	F	9	no	no
12	35	F	7	yes	yes
13	25	F	7	no	yes
14	19	F	2	no	no
15	47	F	19	no	yes

M: male; F: female

AG : Aminoglycoside

Figure 1: Mitochondrial DNA sequence with substitution of a guanine for the adenine at position 1555.

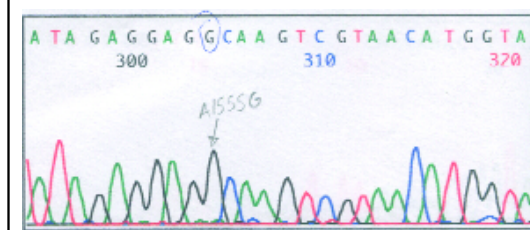


Table II: Severity of hearing impairment in the 15 study patients

	Patients(%)
Mild	2 (13.3%)
Moderate	2 (13.3%)
Severe	4 (26.6%)
Profound	4 (26.6%)
Cophose	3 (20.0%)

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Aminoglycoside ototoxicity exists as two variants, one dose-dependent and the other dose-independent. Ototoxicity in patients with the A1555G mutation is dose-independent. All 4 patients exposed to aminoglycosides in our study had permanent, progressive, and bilateral SNHI. The A1555G mutation increases the similarities between human 12S rRNA and its bacterial 16S homolog susceptible to aminoglycosides. The result is increased binding of the aminoglycoside to human 12S rRNA. However, according to this hypothesis, cochlear damage occurs only upon exposure to aminoglycosides. A reasonable assumption is that the A1555G mutation leads to a major respiratory deficiency (independently from aminoglycoside exposure) related to the primary mitochondrial translation defect and responsible for a decrease in ATP production by cochlear cells (hair cells and/or stria vascularis). This ATP decrease would dramatically affect ion pump function and therefore ion balance, which is crucial to hearing function. In cell types with high oxidative phosphorylation requirements, or in situations of energetic stress, the 50% decrease in mitochondrial protein synthesis observed with the A1555G mutation may have catastrophic effects on the cell [8,30].

In our study, all 4 patients with a history of aminoglycoside exposure had profound hearing loss. This raises the issue of the need for routine mtDNA A1555G screening before aminoglycoside administration. Given that over half the patients in our study had the mutation, we suggest that patients should be asked about their personal and family history of hearing impairment and that a pedigree should be established. When the pedigree suggests matrilineal inheritance of the hearing impairment, aminoglycoside use should be avoided if possible, and preference should be given to other classes of antibiotics. When aminoglycoside use is highly desirable, screening for the mutation should be performed if the test is available.

The true prevalence of the mtDNA A1555G mutation is not known. Several screening options exist: routine screening at birth, screening before aminoglycoside use, screening before aminoglycoside use in patients with familial SNHI, or screening in patients with familial SNHI and a pedigree suggesting matrilineal inheritance. Further data on the prevalence of the A1555G mutation and evaluation of the cost-effectiveness of each strategy are needed.

The ototoxic effects of aminoglycosides per se and the effects of the A1555G mutation involve different mechanisms, which converge to cause death of the ciliated cochlear cells. Hearing loss can occur in the absence of aminoglycoside use in patients with the A1555G mutation. Thus, in our study 4 of the 8 patients with the muta-

tion had no known exposure to aminoglycosides

CONCLUSION

Maintaining a high level of clinical suspicion is essential to the diagnosis of SNHI associated with the mtDNA A1555G mutation. Establishing the diagnosis has major implications for prevention: genetic counseling can be offered to females with the mutation, and both males and females with the mutation but normal hearing can be warned against aminoglycoside use.

Before prescribing aminoglycoside therapy, physicians should ask about a personal and familial history of hearing loss. A pedigree should be established for patients with familial hearing loss. In patients with the A1555G mutation, aminoglycoside use causes bilateral, profound, dose-independent SNHI. The mutation is associated with early-onset progressive SNHI in the absence of aminoglycoside use.

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